Differential patterns of apoptosis in response to aging in *Drosophila*

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Several lines of evidence suggest that programmed cell death may play a role in the aging process and the age-related functional declines of multicellular organisms. To pave the way for the use of *Drosophila* to rigorously test this hypothesis in a genetic model organism, this work examines the pattern of apoptosis in the adult fly during aging. The analysis across the lifespan of caspase activity and DNA fragmentation shows that apoptosis occurs in adult flies at all ages and that it is linked to physiological age. The results establish that under normal conditions, fly aging is coupled with a lifelong gradual increase of apoptosis within muscle cells and an activation of apoptosis in fat cells of old flies. The nervous system does not show signs of apoptosis. These time- and tissue-specific changes indicate that aging influences the levels and the nature of the cells that commit to apoptosis. The comparison with the apoptotic response to starvation and oxidative stresses strongly suggests that the lifelong increase in flight and leg muscles results from the accumulation of oxidative damage associated with aging. This finding presents an attractive mechanism to account for the decline of locomotor functions and muscle loss in the elderly and opens the way for the genetic analysis of sarcopenia in *Drosophila*.

oxidative stress

Despite the tremendous interest in the potential role of apoptosis in aging and age-related diseases, the precise relationships among these processes remain to be established. Advanced age is known to influence death-inducing signals (1–5), and, therefore, aging has the potential to strongly affect multiple aspects of the apoptotic response in a tissue-specific manner. The critical role of apoptosis in tissue homeostasis may have significant implications during aging. In mitotic tissues, apoptosis maintains overall control of immunocompetent cells and also serves as a protective mechanism against age-associated tumorigenesis (6, 7). In these tissues, the effects of aging on apoptosis differ among different cell types or genetic backgrounds (6, 8, 9). In postmitotic tissues, however, aging is associated with a general enhancement of apoptosis, and this trend seems to be essential for the removal of damaged or dysfunctional cells (6, 7). These considerations suggest that a decline of the apoptotic response in mitotic tissues may decrease longevity as a consequence of a higher incidence of cancer or autoimmune diseases, whereas an increase of the apoptotic response in postmitotic tissues may diminish tissue function by removing nonreplaceable cells.

Although a causal link between aging and apoptosis presents an attractive hypothesis, a mechanistic relationship between these processes remains to be demonstrated. Genetic model organisms such as *Caenorhabditis elegans* and *Drosophila* provide a unique opportunity to address this issue. They share with mammals the molecular components regulating or executing apoptosis (10). These components can be manipulated one at a time and in specific tissues to evaluate the impact of a localized alteration of the apoptotic response on the health and longevity of the whole organism. However, such studies require *a priori* knowledge of the natural progression of apoptosis that takes place during normal aging. Extensive microscopic examination during *C. elegans* aging did not reveal any cells undergoing apoptosis (11, 12). Therefore, it is not surprising that the prevention of apoptosis by a caspase mutation did not modify longevity (12). Extensive analysis of the cellular and intracellular changes occurring during *Drosophila* aging indicates tissue-specific alterations, some of which are clearly associated with cell losses (13–15). However, these studies did not determine whether cells are eliminated through apoptotic cell death or necrotic cell death. This report investigates caspase-dependent apoptosis during normal *Drosophila* aging.

Materials and Methods

***Drosophila* Strains and Culture.** The wild-type Canton-S strain, the w^{1118} strain, and the GAL4 enhancer-trap strains w[*];P{w[+mW.hs] = GawB}how[24B], w[*];P{w[+mW.hs] = GAL4-da.G32}UH1 were obtained from the Bloomington Stock Center, Indiana University. The GAL4 enhancer-trap strain w[*];P{w[+mW.hs] = GawB}D42 was a gift from G. Boulianne (Hospital for Sick Children, Toronto). The GAL4 enhancer-trap strains w[*];P{w[+mW.hs] = GawB}DJ634, w[*];P{w[+mW.hs] = GawB}DJ651 and w[*];P{w[+mW.hs] = GawB}DJ694 were previously described and are available from the Bloomington Stock Center (16, 17). All GAL4 strains were crossed with w^{1118}, and the resulting progeny heterozygous for the GAL4 transgene insertion was used.

Age-synchronized cohorts were obtained by emptying cultures and collecting newly emerged flies within 48 h. Collection was done in <2 min under nitrogen anesthesia. Cohorts were maintained at 25°C on fresh *Drosophila* food (0.01% molasses/8.2% cornmeal/3.4% killed yeast/0.94% agar/0.18% benzoic acid/0.66% propionic acid) by changing the food every 3–4 days.

RT-PCR. RNA was extracted from 15 adult flies by using the RNAqueous kit (Ambion, no. 1912). Residual genomic DNA contamination was removed with the DNA-free kit (Ambion, no. 1906). RNA was quantified by spectrophotometry, and its integrity was confirmed visually by running on Tris-acetate-EDTA 0.7% agarose gel electrophoresis. All RT-PCR reactions were performed by using Ready-to-Go RT-PCR Beads (Amersham Pharmacia, no. 27-9267-01). Each reaction contained 0.3 μg of polyd(T), 400 nM primers, and 2 μl of the nucleic acid preparation of interest at varying concentrations in a final volume of 50 μl. Reactions with genomic DNA were systematically performed as a positive control for the PCR. When possible, the specificity of each reaction was tested by using primers surrounding at least one intron, allowing for the RNA- and DNA-derived fragments to be differentiated by size. When no introns lay between primers, specificity was tested by using negative control reactions in which the initial 42°C reverse transcription cycle was omitted, thereby eliminating any fragment amplification of RNA

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Abbreviations: AMC, 7-amido-4-methylcoumarin; DEVD, asp-glu-val-asp.

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origin. All tests used the following conditions in a Biometra thermal cycler: a single 30-min reverse transcription cycle at 42°C and a single pre-PCR cycle at 95°C followed by a variable number of cycles (n) of 30 seconds of denaturation at 95°C, 1 minute at a variable hybridization temperature (D), and a variable time (E) for elongation at 72°C. Reactions were ended with a single 10-min post-PCR cycle at 72°C. The conditions for each gene of interest are listed as follows: STRICA, D = 56°C, E = 2 min, sense 5′-TTACCGCCTCTTTAGTTTGTGAGC-3′, antisense 5′-CCCAGGATGTGAAGGTCTTGC-3′, n = 45; DRICE, D = 58.8°C, E = 1.5 min, sense 5′-CGGCAAAC-CCAAGTTGTTCTTC-3′, antisense 5′-GCAGTGGCAC-CAATCTCGTC-3′, n = 32; DCP-1, D = 59.1°C, E = 2 min, sense 5′-TGACCGACGAGTGCGTAACC-3′, antisense 5′-TAACGAATGTAAGCAGGGTGAGC-3′, n = 32; HID, D = 67°C, E = 1.5 min, sense 5′-TTTGTCGTTCTCGCTCCAC-CTG-3′, antisense 5′-ATCAACACCGCAGCCAATGC-3′, n = 32; GRIM, D = 57.9°C, E = 1.5 min, sense 5′-GTCGTCCT-CATCGTTGTTCTGAC-3′, antisense 5′-CCATCGCCTATT-TCATACCCG-3′, n = 32; DIAP-1, D = 60°C, E = 2 min, sense 5′-TCCTCCGATGAGAGTGATGTCTG-3′, antisense 5′-ATGGTCGCCCAACTGTCCAC-3′, n = 32.

Caspase Activity. The caspase assay is based on the hydrolysis of the peptide substrate Ac-asp-glu-val-asp-7-amido-4-methylcoumarin (Ac-DEVD-AMC), resulting in the release of the fluorescent 7-amido-4-methylcoumarin (AMC). This assay was performed by using a previously described procedure with some modifications (18). Five heads, two thoraces, or two abdomens were homogenized in 40 μl of lysis buffer (50 mM Hepes, pH 7.5/100 mM NaCl/1 mM EDTA/0.1% CHAPS/10% sucrose/5 mM DTT/0.5% Triton X-100/4% glycerol) and centrifuged at 13,000 × g for 5 min at 4°C. The supernatant was used to quantify caspase activity. Ten microliters of head or thorax extracts (≈2 mg/ml protein) or 3 μl of abdomen extracts (≈3 mg/ml protein) were incubated for 1 h at 27°C with 25 mM Ac-DEVD-AMC (Sigma no. A-1086) in lysis buffer with a final reaction volume of 50 μl. The specificity of the detection was controlled in a duplicate reaction pretreated for 15 min at 22°C with 2.5 mM Ac-DEVD-CHO inhibitor (Sigma no. A-0835). The fluorescence of this control reaction was subtracted from the test reaction. AMC fluorescence was determined by using a Spectra Max Fluorescent Microplate Reader (Molecular Devices) with the excitation and emission set at 360 nm and 460 nm, respectively. The concentration of the AMC released was calculated by using an AMC standard curve ranging from 100 nM to 20 mM. Protein concentrations in the various extracts were measured with a Bio-Rad protein assay dye reagent (no. 500-0006). Caspase activity was expressed as nanomoles of AMC per second per milligram of protein.

TUNEL Labeling. Apoptosis-induced DNA fragmentation was detected by labeling free 3′-OH termini with FITC-labeled dUTP by using a protocol derived from procedures described in refs. 19–21. Flies were collected under nitrogen, embedded in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA, no. 4583) and cryosectioned. Frozen sections were fixed with Mirsky's fixative (National Diagnostics, nos. HS-102a and HS-102b) for 10 min at room temperature. The sections were rinsed with PBS (137 mM NaCl/2.68 mM KCl/10.14 mM Na_2HPO_4/1.76 mM KH_2PO_4, pH 7.4) three times, incubated in permeabilization solution (0.1% Triton X-100 in PBS) for 10 min, then rinsed three times in PBS. After equilibration with 200 μl of terminal deoxynucleotidyltransferase (TdT) buffer (25 mM Tris·HCl, pH 6.6/0.2 M potassium cacodylate/0.25 mg/ml BSA/1 mM cobalt chloride) for 5 min at room temperature, the slides were incubated in 200 μl of TdT buffer, supplemented with 60 units of terminal transferase (Roche Applied Sciences, Mannheim, Germany, no. 3333566) and 5 μM fluorescein-12-dUTP (Roche Applied Sciences, no. 1373242) at 37°C for 1 h. The reactions were terminated with TB buffer (0.3 M NaCl/0.03 M sodium citrate) for 15 min at room temperature. The sections were rinsed with PBS three times and mounted in 70% glycerol containing 2.5% DABCO (Sigma, no. D-2522). Images were captured on a Zeiss Axioplan2 Imaging Microscope with a Leica DC 500 high-resolution digital camera and the OPENLAB imaging software (Improvision, Lexington, MA).

Results

Many Apoptosis-Related Genes Are Expressed During *Drosophila* Aging. Apoptosis is a very well characterized process, which depends on and is modulated by the activity of many genes. Hence, it is expected that for apoptosis to occur during aging, the molecular components of the apoptotic machinery must be present in adult flies as they age. Consequently, it is predicted that the genes encoding these components must be transcribed during adult stages, because most of them are submitted to protein turnover and the targeting of some of them to proteolytic pathways is intrinsic to the execution of the cell death program (10, 22). Because most studies on apoptosis have been performed during *Drosophila* development, the expression of apoptosis-related genes is unknown during aging. Genome-wide analysis of gene expression did not reveal age-dependent changes in the expression of apoptosis-related genes (23). However, methods for determining whether a gene is expressed are somewhat arbitrary with the single-channel microarray technology used in this study. Whatever molecular signals lead to the activation of caspase-dependent apoptosis, the signal transduction pathways converge to trigger caspases by acting on few positive and negative regulators. We therefore chose to examine the expression of genes encoding caspases (*drice*, *strica*, and *dcp-1*) or positive (*hid* and *grim*) and negative (*DIAP-1*) downstream regulators known to physically interact with caspases. RT-PCR analysis of RNAs extracted from 20-, 30-, and 40-day-old flies demonstrates that these genes are expressed at all ages with the exception of *strica*, which could not be detected past 20 days in males (Fig. 1 B and C; see also Fig. 5, which is published as supporting information on the PNAS web site). The same results were obtained with different strains to exclude a possible influence of the genetic background on the expression of these genes (data not shown).

Caspase Activity Is Differentially Regulated in Various Body Parts. Once the presence of most components of the apoptotic machinery is established, the next logical step is to test whether apoptosis can be detected during aging. Because the activation of caspases is an early nonreversible hallmark of cells committed to die, a caspase Asp-Glu-Val-Asp (DEVD)-based assay is routinely used to detect and quantify apoptosis. Although *Drosophila* caspases display different substrate preferences, DEVD can report the activity of four of the five caspases with known substrate(s) (see Fig. 6*A*, which is published as supporting information on the PNAS web site). VDVAD and IETD-based assays were also performed to complement the DEVD assay and identify the caspase(s) most likely to contribute to the DEVD activity (see Table 1, which is published as supporting information on the PNAS web site). Cellular extracts were prepared from heads, thoraces, and abdomens to determine the contribution of each body part as well as the contribution of major tissue types. The majority of the head extracts is contributed by the nervous system (brain) with minor contributions from fat and muscles. Thorax extracts are mainly composed of muscles, and the nervous (thoracic ganglion) and digestive (cardia, ventriculus, and crop and salivary glands) systems. Abdominal extracts consist mostly of digestive (ventriculus, crop, anterior intestine, and rectum), reproductive, and adipose systems. A

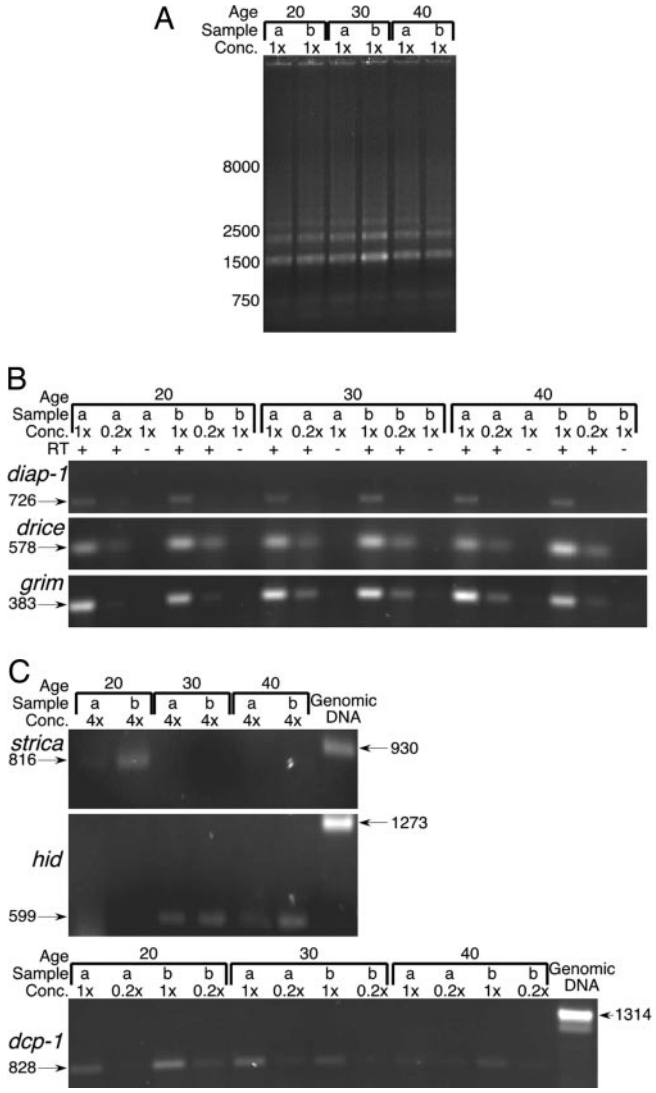

Fig. 1. Expression of apoptosis-related genes with increasing age. Concentration represented as 1× = 520 ng RNA. (*A*) Example of RNA samples, with two extractions (a and b) performed on males aged to 20, 30, and 40 days. Marker sizes indicated at left of gel image (base pairs). (*B* and *C*) RT-PCR analysis of sets of two RNA extracts (a and b) taken from males at varying ages, shown in days, with expected band size indicated (base pairs) at left with arrow. (*B*) Size of genomic DNA fragment equals mRNA band, therefore negative controls performed as lack of initial 30 min reverse transcription step, indicated (−) after concentration. (*C*) Positive control genomic DNA band size indicated (base pairs) at right with arrow.

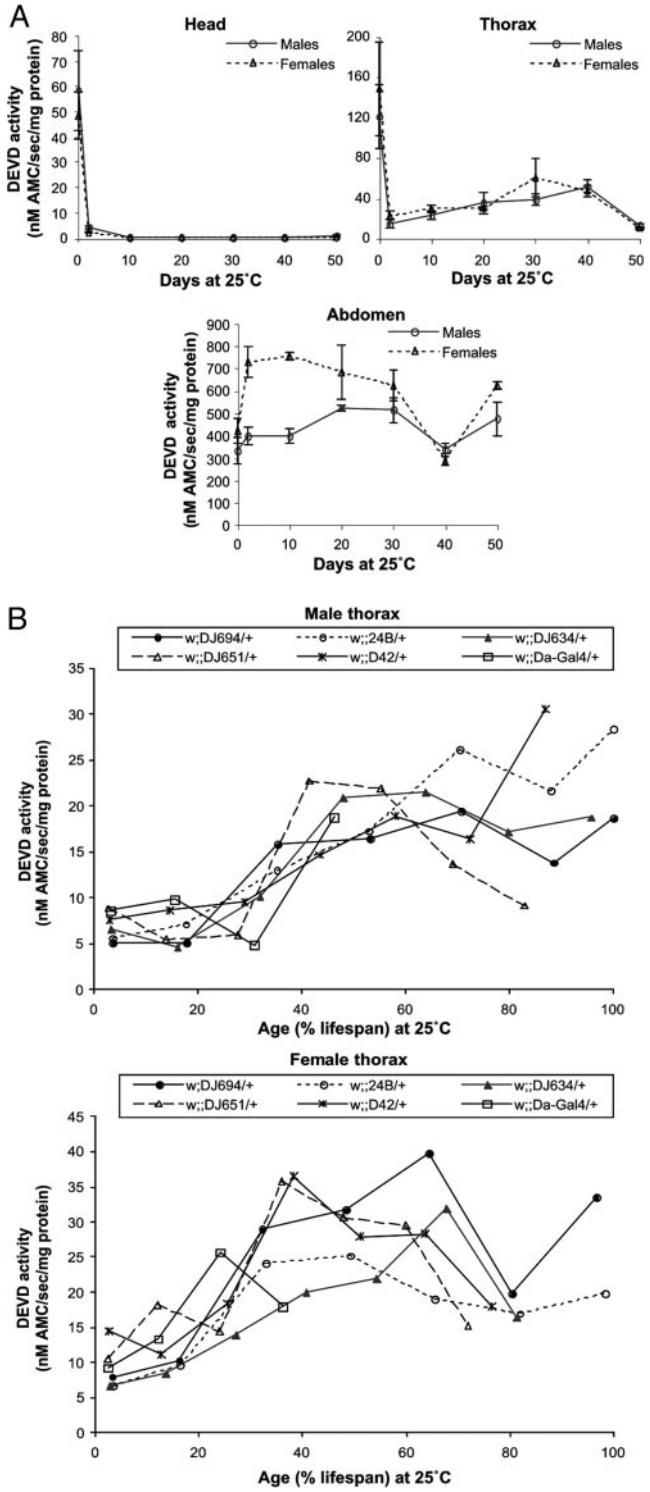

Fig. 2. DEVD activity as a function of age. (*A*) Contribution of various body parts. DEVD activity was quantified in head, thorax, and abdomen of freshly eclosed (<2 h), 2, 10, 20, 30, 40, and 50 days w^{1118} males (solid line) and females (broken line). Data are shown as mean ± SE ($n = 3$). (*B*) DEVD activity in different genetic backgrounds. Age is shown as percent of maximum lifespan for each genotype. Each point represents the mean of at least three samples. Caspase activity in both male and female thorax significantly increases with age.

relatively high caspase activity is detected in the head of freshly emerged adults (Fig. 2*A*). This activity decreases very quickly and is barely detectable in 2-day-old animals, after which the assay does not detect any measurable activity. This observation is consistent with the known involvement of apoptosis in the remodeling of the nervous system during late metamorphosis and the first few days of adulthood (24–26). This phenomenon is also revealed in thorax extracts where high activity is observed in newly emerged adults before decreasing 7- to 8-fold after 2 days of age. Afterward, in contrast with head extracts, significant amount of caspase activity not only remains at all ages examined, but also significantly increases with advancing age. The decrease at the oldest age is not consistently observed (Fig. 2*B*) and is not statistically significant (see Table 2, which is published as supporting information on the PNAS web site), illustrating the sampling bias inherent to old populations where older individuals are survivors that account for a small percentage of the original population. The highest caspase activity is found in

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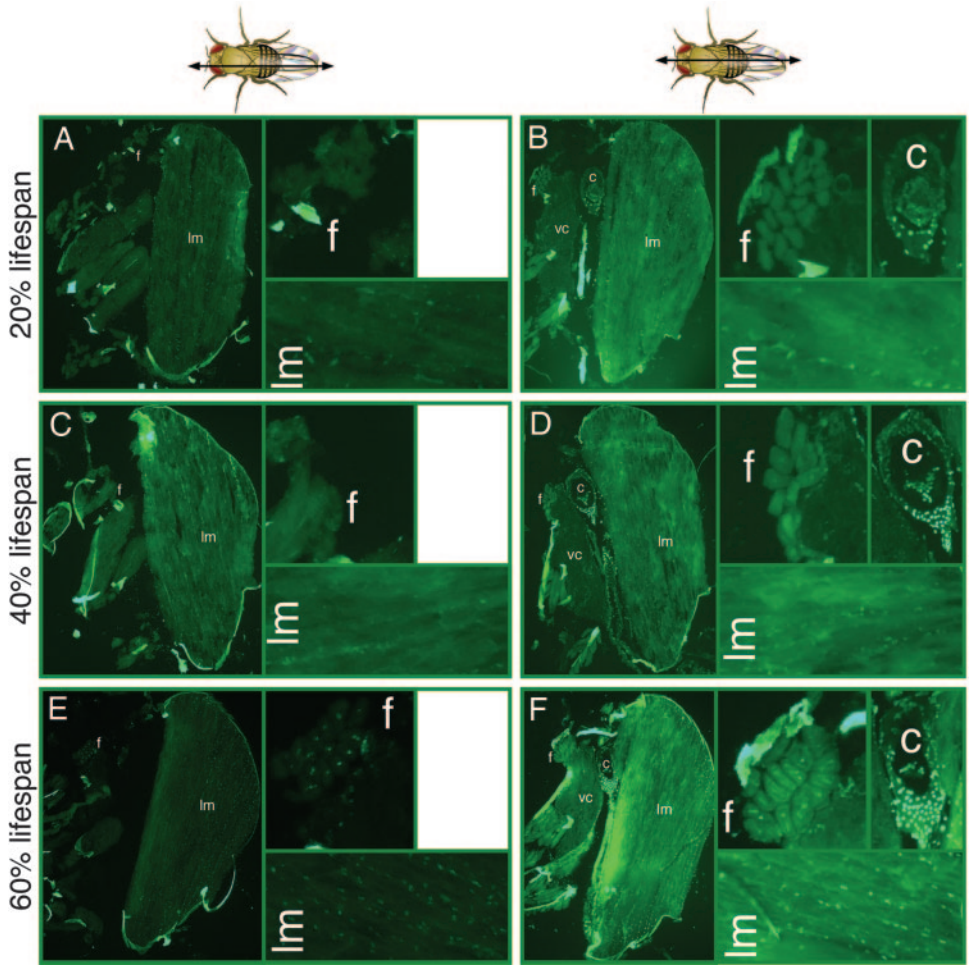

Fig. 3. DNA fragmentation with increasing age. Representative TUNEL images show DNA fragmentation in the thorax of males at age 20% (*A* and *B*), 40% (*C* and *D*), and 60% (*E* and *F*) of lifespan. The fly diagrams at the top indicate the position of the sagital section in the pictures below. *Inset* shows the corresponding labeled area (c, f, and lm) at higher magnification. c, cardia; f, fat; lm, longitudinal muscle; vc, ventral nerve cord.

abdomen extracts. Unlike the other extracts, there is a significant increase in the abdomen of females from emergence to 2 days of adulthood. Although not statistically significant, a similar trend is visible in males. Thereafter, there are no significant changes until 40 days, at which time a significant decrease is observed in females, with a similar trend in males. This observation is in agreement with the importance of apoptosis and caspases in the male and female reproductive systems during gametogenesis and early embryogenesis (27–30). The decrease in older animals is well correlated with the reproductive decline associated with old ages. To exclude the possibility that these observations might reflect an abnormal situation linked to the genetic background of the strain used, similar results were obtained with a wild-type strain (data not shown) and several heterozygous GAL4 driver strains (Fig. 2*B*). The high levels of activity obtained with DEVD as compared with VDVAD and IETD most likely indicate the contribution of downstream caspases (Table 1). Unlike abdomen extracts, the absence of detectable activity with VDVAD and IETD in thorax extracts suggests that the differential profile of DEVD activity between the thorax and the abdomen results from the activity of distinct caspases.

Caspase Activity Changes Are Associated with Physiological Age. To evaluate whether changes in caspase activity associated with chronological age reflect changes on the scale of physiological age, the longevity and caspase activity of heterozygous GAL4 strains has been measured (see Figs. 7 and 8, which are published as supporting information on the PNAS web site). Data for six strains are shown in Fig. 2*B* where time is normalized to the percentage of maximum lifespan. Similar trends are observed between the different strains, indicating that the length of the lifespan does not affect the nature of the changes, but the rate at which they occur. Therefore, the temporal pattern of apoptosis is regulated by physiological age as opposed to chronological age.

DNA Fragmentation Is Differentially Regulated in Specific Tissues. The above results indicate that *Drosophila* aging is associated with dynamic and body part-specific changes in the temporal pattern of caspase activity. The involvement of apoptosis in the modeling of the nervous system and the function of the reproductive system can easily account for the changes in caspase activity observed in the head and abdomen. To confirm this prediction and pinpoint the tissues represented by the changes detected in the thorax extracts, the next logical step is to examine apoptosis *in situ* by TUNEL labeling of DNA fragmentation, another widely used hallmark of apoptosis downstream of caspase activation. In agreement with the previous results and as expected, no staining can be consistently detected in the head after 2 days of adulthood (data not shown; D.W.W., unpublished data) and has been independently reported (31). In the abdomen, the staining is equally distributed between the reproductive

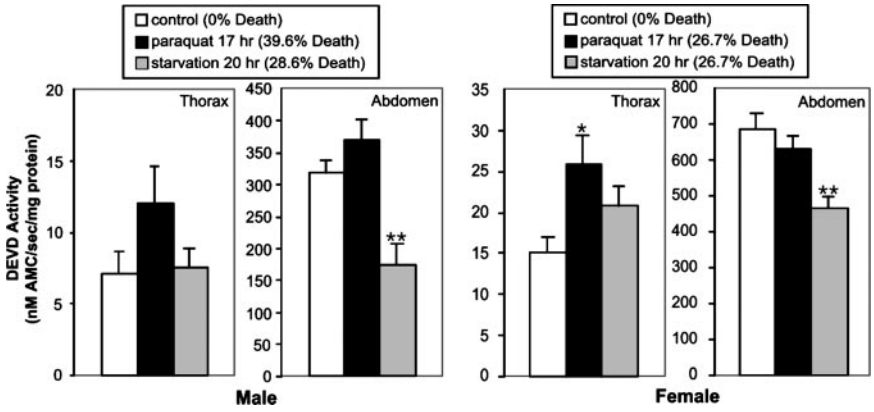

Fig. 4. DEVD activity in response to dry starvation and oxidative stresses. Newly emerged w^{1118} outcrossed to Canton-S were sorted by gender and aged to 4 days at 25°C. Controls were fed 5% sucrose. After 20 h of dry starvation or 3 h of starvation followed by 17 h of exposure to 20 mM paraquat in 5% sucrose, the dead flies were counted and caspase activity was measured in both the thorax and abdomen of controls (open bars), paraquat-treated (black bars), and starved (gray bars) males and females that survived. Paraquat stress increases caspase activity in both male and female thorax. However, starvation significantly reduces caspase activity in both male and female abdomen. Data are shown as mean plus SE ($n = 10$ for each group). *, $0.01 < P < 0.05$; **, $P < 0.01$ compared with control (*t* test).

system and the digestive system (see Fig. 9, which is published as supporting information on the PNAS web site). In young adults (20% of the lifespan), the examination of the thoracic region shows the presence of DNA fragmentation within a small number of nuclei in flight muscles and in the digestive system (Fig. 3 *A* and *B*). In the digestive system, the fluorescent label is located on the outer layer of the cardia and ventriculus, highlighting the scattered muscle fibers associated with the digestive system (32). Some labeling is also detected in the space filled with longitudinal muscle fibers located between the two inner layers of the cardia that form the stomodaeal valve. These observations indicate that apoptosis is restricted to muscle cells early in life. The same tissue distribution is observed at middle age (40% of the lifespan), and the number of positive nuclei and the intensity of the staining are consistent with the elevation of caspase activity detected above with the biochemical assay (Fig. 3 *C* and *D*). In old animals (60% of the lifespan), DNA fragmentation is still observed within the flight muscles and the digestive system (Fig. 3 *E* and *F*). Compared to young animals, there is an obvious increase in the number and brightness of TUNEL-positive nuclei at this age. Old adults also show a more inclusive tissue distribution of the staining. In addition to muscle cells, labeling is observed within fat cells.

Oxidative Stress Induces Apoptosis. Oxidative stress is widely accepted as one of the driving force behind aging. If the lifelong gradual increase of apoptosis in muscle cells reflects the cumulative effects of oxidative damages, one would expect that an acute oxidative stress should increase the level of apoptosis in muscles. Indeed, exposure to hyperoxia (100% O_2) induces widespread apoptosis in the flight muscle of young flies, as indicated by an abundance of TUNEL-positive nuclei and immunoreactivity for the apoptogenic configuration of cytochrome *c* (ref. 33; data not shown). We find similar results by feeding the free-radical generator paraquat. Extracts made from young males and females thoraces exhibit higher DEVD activity (Fig. 4; see also Fig. 10, which is published as supporting information on the PNAS web site). No significant change is detected in the abdomen extracts. However, because of the dual contribution of the reproductive and digestive systems to the activity in abdominal extracts, the DEVD assay is not sensitive enough to uncover changes. Indeed, a TUNEL analysis reveals a strong staining in the digestive tract of paraquat-treated animals, whereas the reproductive system is unaffected (see Fig. 11, which is published as supporting information on the PNAS web site). To establish that the induction of thoracic muscle apotosis is a specific response to oxidative stress rather than a general response to stress, identical analyses were performed after dry starvation treatment. No significant difference is detected between treated and untreated animals in the thorax. In contrast, a significant decrease is observed in the abdominal extracts as expected from the reduction of reproduction associated with nutrient deprivation. Identical results were obtained in older individuals (see Fig. 12, which is published as supporting information on the PNAS web site).

Discussion

Apoptosis is a genetically controlled cell death process essential for development and homeostasis by removing unwanted, damaged, or harmful cells. Several observations have led to speculation that apoptosis contributes to aging. The inability of a cell to induce apoptosis or an inappropriate activation of apoptosis can both lead to detrimental consequences. The former is associated with cancer and rheumatoid arthritis, whereas the latter is coupled with ischemic heart disease, AIDS, and neurodegenerative diseases. Numerous studies link aging with changes in apoptosis under physiological conditions and in the level of induction after a proapoptotic challenge (6, 34). Because aging is associated with cell loss in the heart, brain, and skeletal muscle, apoptosis is suspected to contribute to the impairment of cardiac function, the decline of brain functions, and sarcopenia. However, a mechanistic relationship between organism aging and apoptosis remains to be established. Toward addressing this issue with a genetically tractable organism, this study examines the temporal and spatial pattern of apoptosis during *Drosophila* aging.

Our results demonstrate that most genes encoding critical components of the apoptotic machinery are expressed in adult flies during aging. Interestingly, the proapoptotic genes *grim* and *hid* appear to be up-regulated in 30 days and older animals. The detection of two apoptotic hallmarks, through caspase and TUNEL assays, confirms that apoptosis does occur in adult flies. As expected from its role in gametogenesis (27–30), this study estimates that roughly half of the apoptotic activity detected in the abdomen is localized in the reproductive system. The time course of this activity is consistent with the maturation, reproduction, and postreproduction phases. In freshly emerged adults, high levels of caspase activity are detected in the nervous system

as anticipated from the involvement of apoptosis in its remodeling during metamorphosis and early adulthood (24–26). The ability to reproduce observations made previously in the nervous and reproductive systems is important because it validates the methodologies and findings from this study. This report shows that apoptosis occurs in other tissues. Importantly, the pattern of apoptosis depends on the age of the animal and the nature of the tissue examined.

The most striking finding indicates that aging influences apoptosis in muscle and fat cells. At all ages examined, apoptotic hallmarks are detectable in muscle cells, indicating that these cells can be committed to apoptosis at any time during aging. In addition, caspase activity and the number of nuclei undergoing DNA fragmentation increases in the thoracic and leg muscles of older animals. The apoptotic properties of these tissues provides an attractive mechanism to account for the decline of locomotor functions (14, 35–37) and muscle degeneration (13, 38) that occur during normal *Drosophila* aging. Similar observations and conclusions have been reported in vertebrates. Skeletal muscle atrophy and the loss of myofibers are associated with sarcopenia: a reduction of muscle mass and strength occurring during normal aging in rodents and humans (8, 39). The rare reports on the incidence of apoptosis in muscles during normal aging did correlate sarcopenia and the loss of muscle cells with elevated DNA fragmentation in rats and humans (40, 41). In this study, the course of apoptosis has been linked to the physiological age of the fly by taking advantage of variations in longevity resulting from the genetic background. The same interpretation can be drawn in rats from the manipulation of longevity by caloric restriction found to attenuate muscle fiber apoptosis (42). The similarity between flies and vertebrates is critical because it predicts that the *Drosophila* model has the potential to shed light on the genetic basis of sarcopenia.

Our analysis of the apoptotic response to starvation and oxidative stresses suggests that the increased apoptosis in the flight and leg muscles results from the accumulation of oxidative damage with advancing age. Different apoptotic properties are observed in the nervous system, where no signs of apoptosis could be detected during normal aging. The nervous system is not simply refractory to apoptosis because DNA fragmentation can be revealed in several fly models of neurodegenerative diseases (31, 43, 44), indicating a higher tolerance or a differential response to oxidative damages rather than the absence of the basic apoptotic machinery.

It is evident that cell types and tissues respond differently to aging and oxidative stress. Fly aging is associated with the preferential increased *hsp70* expression in flight and leg muscles (45). Different pattern of gene expression are observed in the skeletal muscle, brain, and heart during mice aging (46–48). However, it is difficult to predict from gene expression changes the mechanisms that act at the cellular level to ultimately contribute to how a multicellular organism age. Extensive studies of free radical scavenging enzymes have provided one such mechanism. The functional significance of this mechanism has been demonstrated by the fact that the manipulation of these enzymes in the nervous system extends longevity, whereas the same manipulation in muscles has no effects (49). The differential tissue-specific apoptotic properties revealed by this study offer another cellular mechanism to investigate with the power of *Drosophila* genetics.

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